Soil Biology and Biochemistry 116 (2018) 224–236

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Climate warming impacts on soil organic carbon fractions and aggregate stability in a Tibetan alpine meadow

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ARTICLE INFO

Keywords:
Experimental warming
Soil organic carbon
Soil aggregates
Solid-state ^{13}C NMR
Alpine meadow
Tibetan Plateau

ABSTRACT

Alpine meadows in the Tibetan Plateau contain a large amount of soil organic carbon (SOC), which is highly vulnerable to climate change and has thus been a research priority for scientists in recent decades. However, how climate warming influences the composition and stability of SOC remains unclear. In this study, a warming experiment (2010–2015) using open-top chambers was established (with unwarmed control [CK], winter warming [WW], and year-round warming [YW]) to investigate the effects of warming on the contents of SOC and its fractions, chemical composition of SOC, and water stability of aggregates in an alpine meadow located at the Damxung Grassland Station in the northern Tibetan Plateau. Experimental warming had no apparent effect ($p > 0.05$) on SOC content (17.8 ± 1.96, 17.8 ± 1.13 and 17.8 ± 1.09 g kg^{-1} under CK, WW and YW, respectively) in the 0–20 cm soil layer. However, warming significantly ($p < 0.05$) increased soil water-soluble organic carbon (C) content by 46.2% and 69.2%under WW and YW, respectively, and affected SOC chemical composition with decreasing phenol C (by 2.6% under WW, $p > 0.05$; and 8.4% under YW, $p < 0.05$) and increasing carboxyl C (by 11.6% under WW, $p < 0.05$; and 5.0% under YW, $p > 0.05$). Warming decreased ($p > 0.05$) the proportions of macroaggregates (2–0.25 mm) and free microaggregates (0.25–0.053 mm), whereas warming significantly increased the proportion of non-aggregated silt- and clay-sized fractions (< 0.053 mm) by 41.0% and 55.7% under WW and YW, respectively. The variation in the aggregate size distribution resulted in the decline of the mean weight diameter and geometric mean diameter of water-stable aggregates by 5.1% ($p > 0.05$) and 8.5% ($p < 0.05$) under WW and by 8.5% and 6.6% (both $p < 0.05$) under YW, respectively. Of importance, the organic C content in free microaggregates, which provides greater physical protection to stabilize SOC, decreased by 10.9% ($p > 0.05$) under WW and by 22.4% ($p < 0.05$) under YW. The organic C contents of particulate organic matter and silt- and clay-sized fractions inside free microaggregates also significantly decreased under YW by 49.4% and 16.9%, respectively. However, the organic C content in non-aggregated silt- and clay-sized fractions significantly increased under WW and YW by 52.9% and 46.8%, respectively. The results suggest that short-term climate warming did not affect total SOC stocks, whereas it had a positive effect on WSOC, carboxyl C and non-aggregated silt- and clay-associated C and exhibited a negative effect on phenol C, free microaggregates-associated C and water stability of aggregates. These variables may act as sensitive indicators of climate warming in the Tibetan alpine meadows, which will in turn affect grassland ecosystem C fluxes in response to further climate change. The findings of the present study help improve our understanding of the responses of terrestrial ecosystem C cycling to future climate change.

1. Introduction

Soil is the third largest reservoir of carbon (C), next to the lithosphere and the oceans, and stores approximately 1500 Gt in the top 1 m depth (Dlamini et al., 2016; Raheb et al., 2017), equivalent to almost twice the amount of C in the atmosphere and three times that in

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http://dx.doi.org/10.1016/j.soilbio.2017.10.011
Received 20 January 2017; Received in revised form 15 September 2017; Accepted 13 October 2017
Available online 31 October 2017
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the terrestrial biomass (Dlamini et al., 2016). Even small changes in soil C stocks could have a vast impact on atmospheric CO_2 concentration (Muñoz-Rojas et al., 2013). As a consequence, the global average surface temperature has increased by 0.85 °C over the past 130 years (1880–2012) (IPCC, 2013). In China, the average surface temperature has increased by 1.40 °C during 1951–2007 (Ren et al., 2012). Elevated surface temperature can substantially impact global C budgets and produce positive or negative feedbacks to climate change (Wan et al., 2005). Therefore, understanding the response of soil organic carbon (SOC) stocks to warming is of critical importance to evaluate the feedbacks between terrestrial C cycle and climate change.

The extent to which climate warming influences SOC mainly depends on the stability of organic C in soil. Based on the decomposition degree and turnover rate (von Lützow et al., 2007), SOC can be chemically divided into labile or rapidly decomposed fractions [e.g., microbial biomass C (MBC), water-soluble organic C (WSOC), easily oxidizable organic C (EOC), particulate organic C (POC), and light fraction organic C (LFOC)] and resistant or slowly decomposed fractions [e.g., heavy fraction organic C (HFOC), humic acid C (HAC), fulvic acid C (FAC) and humin C (HUC)]. Both labile and resistant SOC fractions have been reported to act as potential indicators of climate change (Steinberg, 2003; Wang et al., 2016; Hu et al., 2017). Although the labile SOC fractions were assumed to be sensitive to climate warming (Song et al., 2012), some studies have found that the resistant SOC fractions had more sensitivity (Knorr et al., 2005) or similar responses to climate warming (Fang et al., 2005).

With respect to the stabilization mechanisms of SOC, it has been generally recognized that SOC could be protected against mineralization by incorporation into aggregates (physical protection), sorption onto clay (chemical protection), biochemical transformation into products that are resistant to microbial attack (biochemical protection), and translocation and storage in the subsoil (Six et al., 2002; Sarkhot et al., 2007). Although there have been some disputes (Fontaine et al., 2007; Dungait et al., 2012), physical protection within stable aggregates has been considered to be one of the major mechanisms of SOC stabilization (O'Brien and Jastrow, 2013; Smith et al., 2014; Chaplot and Cooper, 2015; Zhong et al., 2017). Soil aggregate-associated C has been used as an indicator for evaluating soil C sequestration (Six et al., 2000; Bronick and Lal, 2005). Specifically, hierarchically organized soil aggregates, a key feature of soil where organic matter is a major aggregate binding agent (Tisdall and Oades, 1982), play an essential role in the accumulation and stabilization of SOC by physically reducing the bioaccessibility (O'Brien and Jastrow, 2013; Zhao et al., 2017). In fact, soil aggregate stability is not only a key element of SOC stabilization but also can be used to predict SOC outputs from soils (Chaplot and Cooper, 2015). Chaplot and Cooper (2015) pointed to an increase of C losses by water erosion as soil aggregation decreased but an increase in gaseous emissions as soil aggregation improved. In addition, knowledge of SOC chemical composition is also important and could provide information for understanding the biochemical mechanism of the accumulation and stabilization of SOC at the molecular level (Six et al., 2002; Guo et al., 2016).

Grasslands cover approximately 40% of the world's land surface and store about 10% of the global SOC stock (Suttie et al., 2005). Grasslands are a key constituent of biogeochemical C cycle and can provide vital ecosystem services and goods (Dlamini et al., 2014). SOC in grassland ecosystems is not only critical for climate change but also yields important feedbacks to plant productivity and soil fertility (Dlamini et al., 2016). To date, some studies have examined the impact of climate change on SOC in grassland ecosystems. However, the results of previous studies have been contradictory. For example, warming had positive (Li et al., 2011), negative (Li et al., 2011; Wan et al., 2005), or no effects (Wang et al., 2014; Zhang et al., 2015) on SOC content. Likewise, warming could increase (Belay-Tedla et al., 2009; Zhang et al., 2015), decrease (Zhou et al., 2013), or have no obvious effect (Wang et al., 2014) on MBC content. The lack of consistent responses of SOC to warming is not surprising, given the complexity of key controlling factors for C cycling in different grassland ecosystems (Xu et al., 2012; Zhou et al., 2013). Therefore, more information is required to predict how climate change will affect SOC in grassland ecosystems in the future.

With respect to other grassland ecosystems, alpine grassland ecosystem is more sensitive to climatic change (Zhang et al., 2016). The Tibetan Plateau, a typical alpine ecosystem dominated by alpine grasslands, is the highest (a mean elevation of 4000 m above sea level) and largest (an area of 2.5×10^6 km^2) plateau on earth (Wang, 2016). It is estimated that the Tibetan Plateau contains 30–40 Gt of SOC, which accounts for more than 20% of the SOC storage in China and 2–3% of the global soil C stocks (Shang et al., 2016). Previous studies have demonstrated that the Tibetan Plateau has been subjected to distinct warming in recent decades (Guo and Wang, 2011; Wei and Fang, 2013). The magnitude of warming on the Tibetan Plateau is approximately 0.32 °C greater than that of the global average and that of other regions in China (Liu and Chen, 2000; Ren et al., 2012). It has been predicted that the average surface temperature on the Tibetan Plateau will be 2 °C higher than the global average by 2050 (Thompson et al., 2000). Therefore, how SOC responds to climate warming in the Tibetan alpine grassland ecosystem has become a research priority in recent decades.

Alpine meadow, which accounts for over 40% of the Tibetan Plateau area (Fan et al., 2011), is considered particularly sensitive to climate warming (Jiang et al., 2016). In previous studies, some researchers have explored the effects of experimental warming on SOC content (Li et al., 2011; Wang et al., 2014; Yu et al., 2014; Zhang et al., 2015) in alpine meadows on the Tibetan Plateau. Moreover, some researchers have also examined the impacts of experimental warming on labile SOC fractions (MBC, WSOC) contents (Luo et al., 2009; Rui et al., 2011; Wang et al., 2014; Yu et al., 2014; Zhang et al., 2015). As in other grassland ecosystems, uncertainty remains about how experimental warming affects SOC and its fractions contents in the Tibetan alpine meadow ecosystem. To the best of our knowledge, no studies have explored how experimental warming impacts SOC chemical composition and soil structure stability in the Tibetan alpine meadow. This study conducted warming experiment using open-top chambers (OTCs) to investigate how climate warming affects SOC and its fractions contents, SOC chemical composition, and soil aggregate stability in a Tibetan alpine meadow. The specific objectives of this study were to evaluate: (1) warming impacts on the quantity and quality of SOC with respect to labile and resistant SOC fractions and SOC chemical composition and (2) warming influences on hierarchically organized soil aggregates and their stability. A previous study in this OTCs experiment showed that aboveground biomass significantly decreased during short-term (2 years) warming (Zong et al., 2013). Given that the input of aboveground litter to soil is a major process that regulats the quantity and quality of SOC (Zech et al., 1997; Xu et al., 2013; Cao et al., 2016; Li et al., 2017) and the stability of soil aggregates (Bronick and Lal, 2005; Cao et al., 2016; Li et al., 2017), we hypothesized that SOC and aggregate stability would change because of variation of the balance between C inputs by litter and C losses from mineralization and erosion.

2. Materials and methods

2.1. Study site, experimental design, and soil sampling

Field warming experiments were conducted in a fenced alpine meadow located at the Damxung Grassland Station, Tibetan Plateau (30°51′N, 91°05′E, 4333 m above sea level) (Fig. 1). The mean annual temperature is 1.3 °C, and the mean annual precipitation is 476.8 mm. The growing season extends from May to September, and the freezing period extends from October to next April. The soil is classified as Cambosol (Chinese Soil Taxonomy) or Inceptisol (USDA Soil Taxonomy), with a pH of 6.95 and containing 67.0% sand, 18.2% silt, 14.7% clay, 11.3 g kg^{-1} of organic C, and 1.20 g kg^{-1} of total N in the

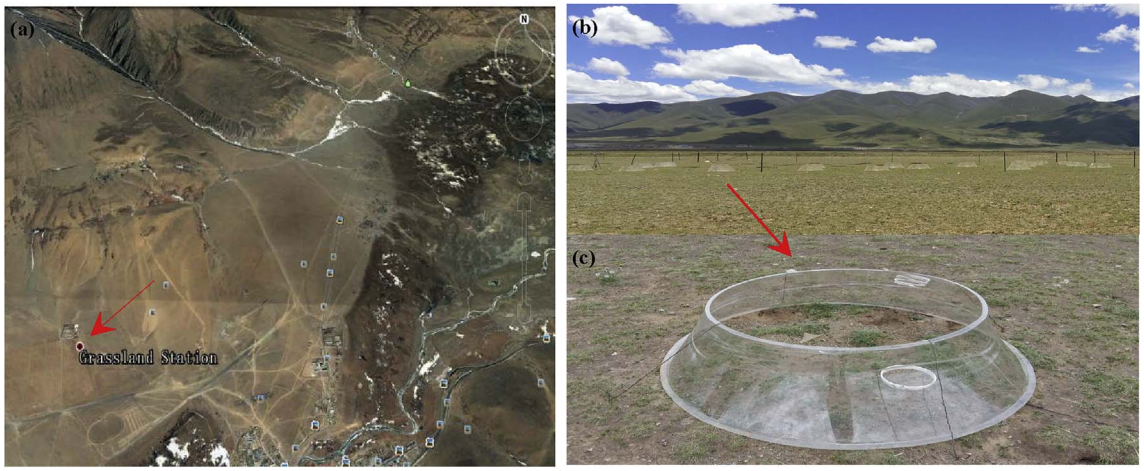

Fig. 1. Google Earth image of the Damxung Grassland Station on the Tibetan Plateau (a) and photographs of the experimental plots (b) and open top chamber (c).

0–15 cm soil layer. According to Dlamini et al. (2016), SOC stocks in this alpine grassland ecosystem are more vulnerable to grassland degradation because of the dry climate (< 600 mm) and sandy (< 20% clay) soil. The vegetation is a *Kobresia*-dominated alpine meadow, and the roots are concentrated in the topsoil (0–20 cm depth).

The field experiments involved three treatments, i.e., unwarmed control (CK), year-round warming (YW), and winter warming (WW). The experimental plots were arranged in a randomized complete block design with five replicates. According to the paradigm of the International Tundra Experiment, we used OTCs as warming device. OTCs are considered to have few unwanted side effects (e.g., temperature extremes, altered light and wind, and site disturbance) (Marion et al., 1997; Hollister and Webber, 2000) and are thus the most prevalent simulation method used to study the responses of alpine ecosystems to experimental warming (Li et al., 2011; Zong et al., 2013; Yu et al., 2014; Seo et al., 2015; Birkemoe et al., 2016; Chen et al., 2016). The conical OTCs used in the present study were made of 3 mm thick polycarbonate plastic, with the opening at the top being 100 cm in diameter, while the bottom is 150 cm in diameter, and the height is 40 cm (Fig. 1). The experimental plots for CK (1 m × 1 m) and YW were established in early July 2010. However, the experimental plots for WW were established in 2012, in which the OTCs were removed from the plots during early May and late September of each year. The plots were separated by approximately 3 m. The effects of warming on soil temperature and moisture at 5 cm depth were synchronously monitored since June 2012 by a HOBO weather station (Onset Inc., Bourne, MA, USA) at 30 min intervals (Zong et al., 2013).

Soil samples were collected at 0–20 cm depth in June 2015. Five soil cores (5 cm diameter) were randomly taken from a 100 × 100 cm area (the approximate area of top opening of OTCs) centered in each plot and then thoroughly mixed to form a single sample. Fresh soil samples were gently broken apart by hand and passed through a 5 mm sieve. Soil samples were then divided into two parts: (1) for the analysis of soil aggregates and (2) for the analysis of SOC and its fractions following further sieving through a 2 mm sieve.

2.2. SOC measurements

SOC content was determined by H_2SO_4-$K_2Cr_2O_7$ oxidation followed by titration with $FeSO_4$ (Lu, 2000). MBC was determined using the chloroform fumigation and extraction method (Brookes et al., 1985). EOC was determined by the $KMnO_4$ (333 mM) oxidation procedure (Blair et al., 1995). Particulate organic matter (POM) was obtained by dispersing soil samples in 5 g L^{-1} $(NaPO_3)_6$ solution for 18 h and then passing them through a 0.053 mm sieve using a flow of distilled water. The material retained on the sieve (POM) was oven-dried (60 °C) and weighed (Cambardella and Elliott, 1992). Light and heavy organic matter fractions were separated by the density fractionation (1.8 g cm^{-3} NaI) method (Chen et al., 2017). Water soluble and humic fractions were obtained by successively extracting soil samples with distilled water and 0.1 M NaOH + 0.1 M $Na_4P_2O_7$ solution, respectively. The humic acid (HA) fraction was isolated from the fulvic acid (FA) fraction by acidifying the alkaline supernatants to pH 1.0, and the alkaline-insoluble residue was the humin (HU) fraction (Wang et al., 2016).

2.3. Soil aggregate separation

Soil aggregates were hierarchically separated according to the procedure described by Yan et al. (2012) and O'Brien and Jastrow (2013) (Fig. 2). In brief, air-dried soil samples (< 5 mm) were first wet sieved into macroaggregates (2–0.25 mm), free microaggregates (0.25–0.053 mm), and non-aggregated silt + clay fractions (< 0.053 mm). The water floatable substances on the 2 mm sieve were removed. No large macroaggregates (> 2 mm) were present in the experimental soil.

The macroaggregates were soaked in distilled water for 10 min, and then poured onto the top of a set of sieves with openings of 0.25 and 0.053 mm mounted on a reciprocating shaker (186 oscillations min^{-1}). After a constant flow of water passed through the 0.25 mm sieve, the materials retained on the 0.25 and 0.053 mm sieve were coarse POM inside macroaggregates (M(*c*)POM) and microaggregates-within-macroaggregates (mM), respectively. The material in the rinse water was silt + clay fractions inside macroaggregates (M-silt + clay).

The free microaggregates were gently inverted in NaI solution (1.85 g cm^{-3}). The light fraction obtained by centrifugation was non-occluded fine POM [Free (f)POM]. The heavy fraction was dispersed in 5 g L^{-1} $(NaPO_3)_6$ solution, and then passed through a 0.053 mm sieve. The material retained on the sieve was POM inside free microaggregates (Fm-POM), and the material in the rinse water was silt + clay fractions inside free microaggregates (Fm-silt + clay). A similar procedure was used to fractionated the mM into non-occluded fine POM [M(f)POM], POM inside mM (mM-POM), and silt + clay fractions inside mM (mM-silt + clay).

2.4. Solid-state ^{13}C NMR analysis

After treatment with 10% HF-HCl solution (Schmidt et al., 1997), solid-state ^{13}C cross-polarization magic-angle-spinning (CPMAS) NMR spectra of soil samples were obtained on an AVANCE III 400 WB spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) at 100.6 MHz with a spinning rate of 8 kHz, an acquisition time of 20 ms,

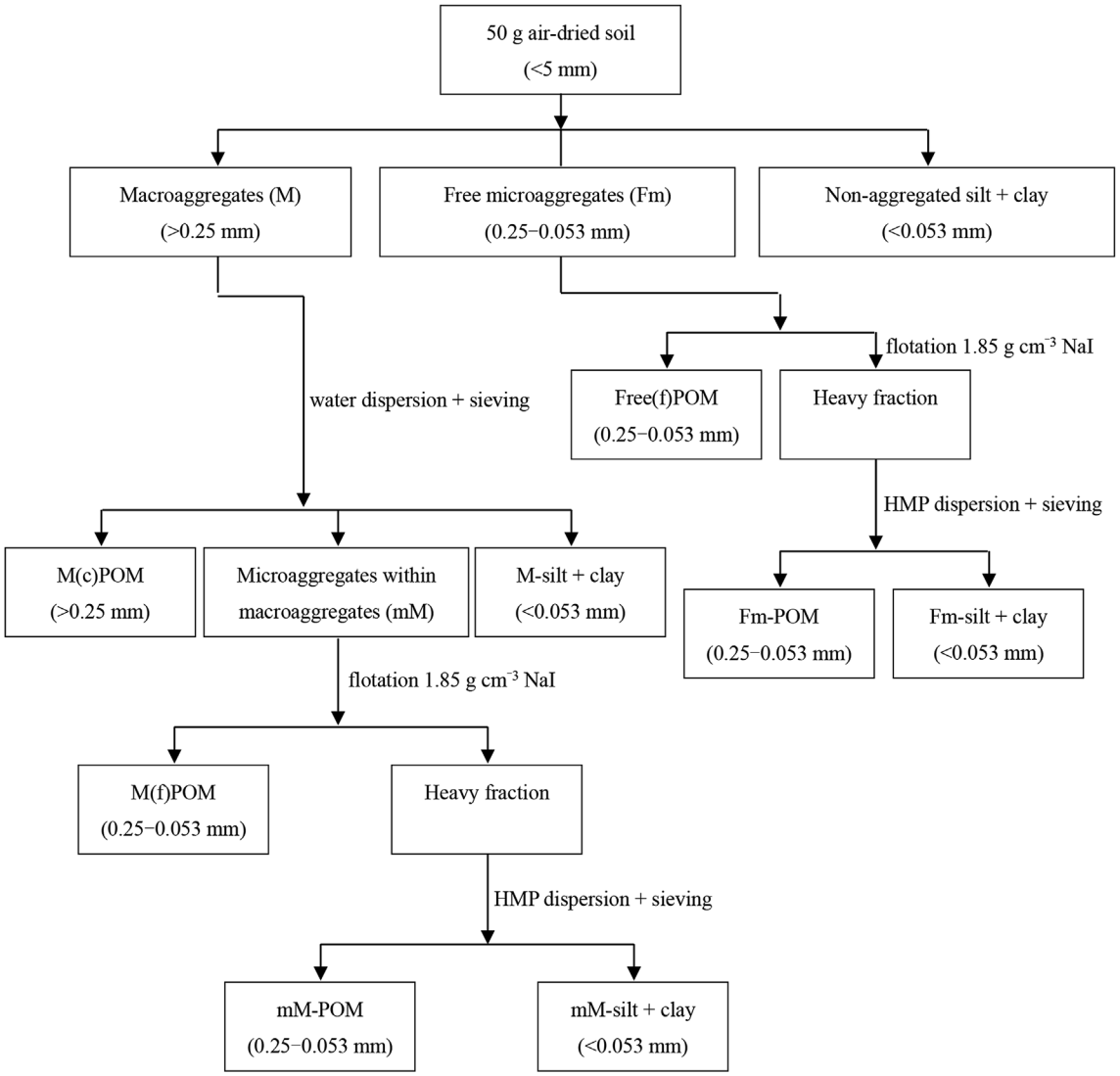

Fig. 2. Fractionation scheme to isolate soil aggregates fractions. M(*c*)POM: coarse particulate organic matter (POM) inside macroaggregates but outside microaggregates; M(f)POM: non-occluded fine POM inside macroaggregates but outside microaggregate; mM-POM: POM inside microaggregaters-within-macroaggrgates; M-silt + clay: silt- and clay-sized fractions inside macroaggregates; mM-silt + clay: silt- and clay-sized fractions inside microaggregaters-within-macroaggrgates; Free (f)POM: non-occluded fine POM not inside any aggregates; Fm-POM: POM inside free microaggregates; Fm-silt + clay: silt- and clay-sized fractions inside free microaggregates (adapted from Yan et al., 2012; and O'Brien and Jastrow, 2013). HMP: sodium hexametaphosphate.

a recycle time of 3 s, and a contact time of 2 ms. Chemical shift values were externally referenced to the methylene resonance of the adamantane standard at 38.4 ppm. Semi-quantification was performed by integration using MestReNova 5.3.1 software (Mestrelab Research S.L., Santiago de Compostela, Spain).

2.5. Statistical analysis

All statistical tests were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The differences among treatment means were determined using one-way analysis of variance (ANOVA) with least significant difference (LSD) test. The significance was set at the $p < 0.05$ level.

3. Results

3.1. Changes in temperature, moisture, and aboveground biomass

Meteorological data from June 2012 to May 2015 showed that experimental warming increased both air and soil temperature but decreased soil moisture content (Fig. 3). In comparison with CK, warming increased the mean annual air and soil temperature (at 5 cm depth) by 1.9 °C and 1.3 °C, respectively, but decreased soil moisture (at 5 cm depth) by 4.70% (v/v) in YW. During three years of WW, the average soil temperature increased by 1.3 °C, whereas soil moisture decreased by 4.80% (v/v). Moreover, we found that soil moisture holding capacity was not significantly different ($p > 0.05$) among the three treatments (55.3, 53.3 and 56.0% in CK, WW and YW, respectively).

At the end of the growing season in 2015, we observed that plant aboveground biomass was lower in WW and YW (124.9 and 84.9 g m^{-2}, respectively) than in CK (131.4 g m^{-2}). The difference between CK and YW was significant ($p < 0.05$).

3.2. Changes in SOC and (labile and resistant) SOC fractions contents

Compared with CK, WW and YW had no significant effect ($p > 0.05$) on SOC content (Fig. 4). Similarly, MBC, EOC, POC, LFOC, HFOC, HAC, FAC and HUC contents were not significantly different in WW and YW in comparison with CK. In contrast, WSOC content

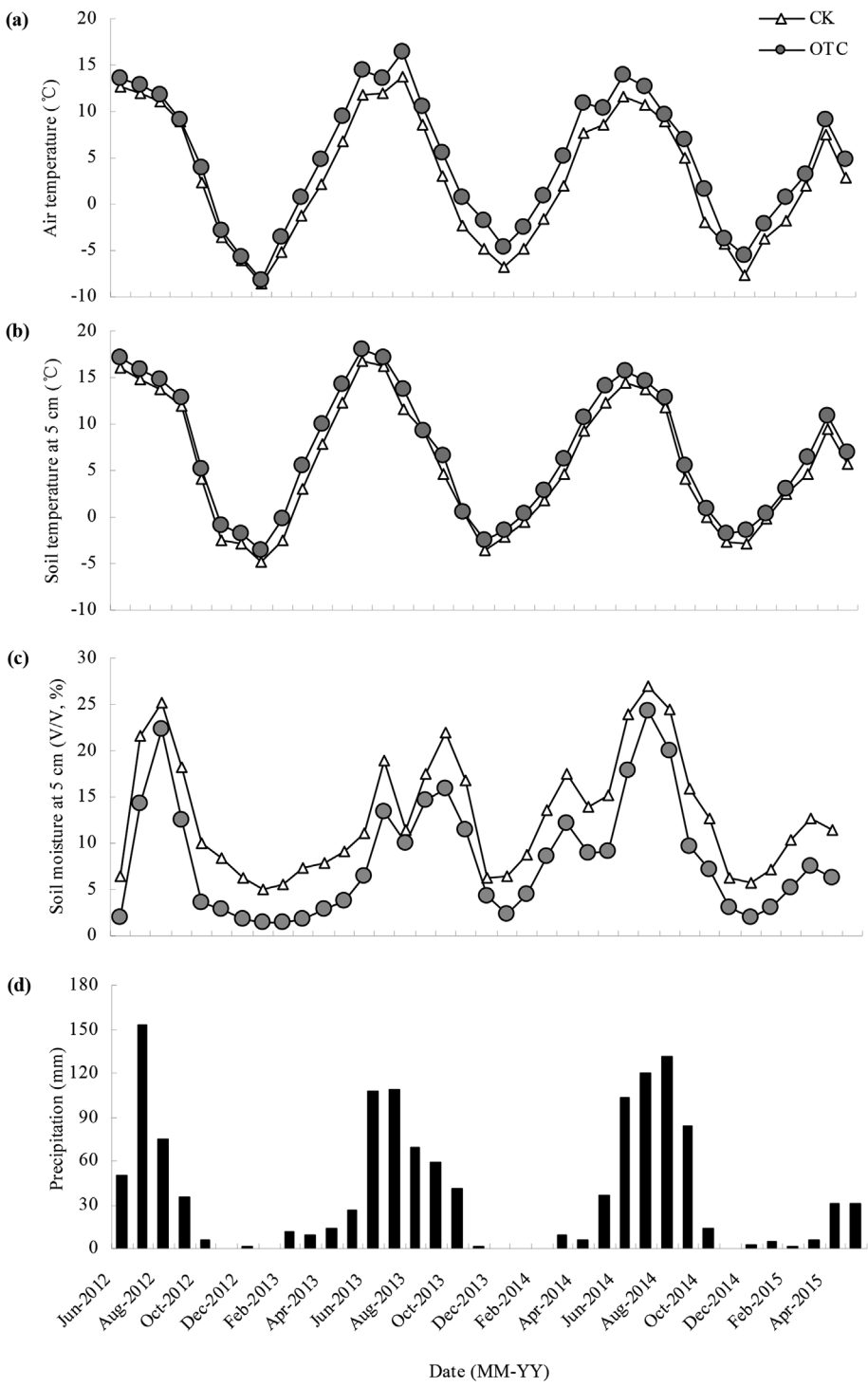

Fig. 3. Average air temperature (a), soil temperature (b), soil moisture at 5 cm depth (c) and precipitation (d) in the open top chambers (OTCs) and control (CK) during the experiment period from June 2012 to May 2015.

significantly increased compared with CK, by 46.2% in WW and 69.2% in YW. There was no significant difference in SOC and its fractions contents between WW and YW.

3.3. Changes in SOC chemical composition

The ^{13}C CPMAS NMR spectra of bulk soil samples were identified as alkyl C (0–50 ppm), O-alkyl C [divided into methoxyl C (50–60 ppm), carbohydrate C (60–95 ppm), and di-*O*-alkyl C (95–110 ppm)], aromatic C [divided into aryl C (110–145 ppm) and phenolic C (145–160 ppm)], and carboxyl C (160–190 ppm) (Fig. 5).

Across all treatments, the proportion of O-alkyl C (38.6–40.7%) was the largest, followed by alkyl C (22.6–23.6%), aromatic C (23.9–24.7%) and carboxyl C (12.1–13.5%) (Fig. 6). Moreover, O-alkyl C was dominated by carbohydrate C (22.3–23.4%), and aromatic C was dominated by aryl C (16.4–16.8%). Compared with CK, alkyl C, methoxyl C, carbohydrate C, di-*O*-alkyl C and aryl C in soil were not significantly different in WW and YW. In contrast, phenol C decreased by 2.6% ($p > 0.05$) in WW and 8.4% ($p < 0.05$) in YW, whereas carboxyl C increased by 11.6% ($p < 0.05$) in WW and 5.0% ($p < 0.05$) in YW compared with CK. The decrease of phenols suggested that warming resulted in destabilization of old or resistant organic C. The ratios of alkyl C/O-alkyl C, aliphatic C/aromatic C and hydrophobic C/hydrophilic C were not significantly different among the three treatments.

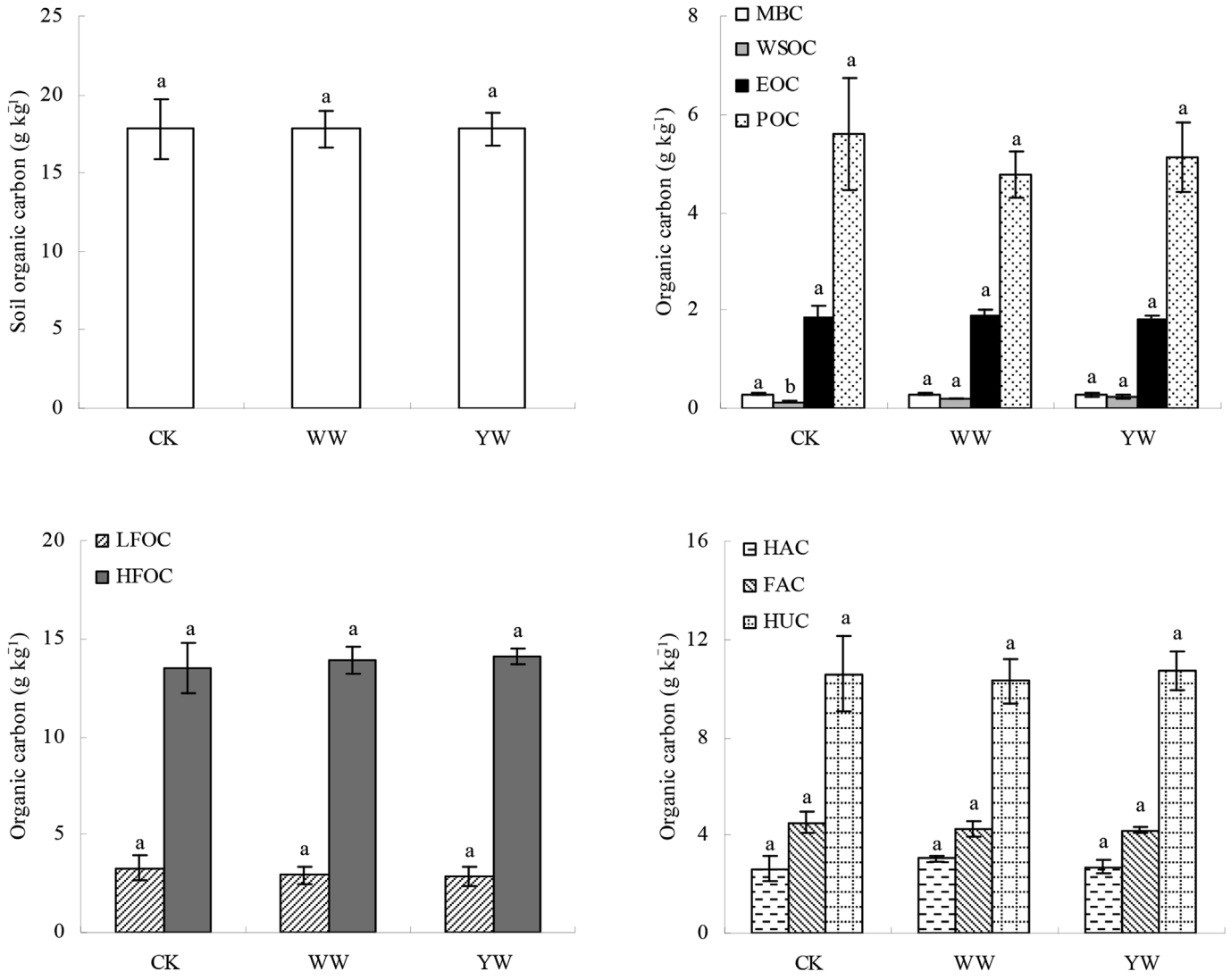

Fig. 4. Contents of soil organic carbon and its fractions under different treatments. Vertical bars represent standard deviation of the mean (n = 5). Different letters indicate significant differences among treatments (p < 0.05). CK: unwarmed control; WW: winter warming; YW: year-round warming. WSOC: water-soluble organic carbon; MBC: microbial biomass carbon; EOC: easily oxidizable organic carbon; POC: particulate organic carbon; LFOC: light fraction organic carbon; HFOC: heavy fraction organic carbon; HAC: humic acid carbon; FAC: fulvic acid carbon; HUC: humin carbon.

Moreover, no significant differences for the proportions of various C functional groups were detected between WW and YW.

3.4. Changes in soil aggregate-associated C contents

Macroaggregate-associated organic C had the highest content (6.38–7.32 g kg^{-1}) in all three treatments, followed by free microaggregates (4.57–5.89 g kg^{-1}) and non-aggregated silt + clay fractions (3.25–4.97 g kg^{-1}) (Fig. 7). Moreover, M-silt + clay fractions, Fm-silt + clay fractions and mM-silt + clay fractions exhibited the highest organic C contents in macroaggregates, free microaggregates and mM, respectively.

The different C fractions of the hierarchical aggregates exhibited distinct responses to climate warming. Compared with CK, WW and YW had no significant effect on macroaggregates-associated organic C content, whereas C associated with non-aggregated silt + clay fractions significantly increased by 52.9% in WW and 46.8% in YW. Free microaggregate-associated C was not significantly different in WW than in CK but was 22.4% (p < 0.05) lower in YW than in CK. Moreover, a significant decrease in the organic C content of Fm-POM (49.4%, p < 0.05) and Fm-silt + clay fractions (16.9%, p < 0.05) was detected in YW compared with CK.

Compared with WW, the organic C contents of mM-silt + clay fractions and free (f)POM was 34.2% and 91.7% (p < 0.05) lower in YW, respectively, suggesting that C associated with mM-silt + clay fractions and free microaggregates were preferentially lost with greater warming intensity.

3.5. Changes in soil aggregate size distribution and aggregate stability

Across all experimental treatments, macroaggregates exhibited the largest proportion (42.0–46.3% of the total soil mass), followed by free microaggregates (37.4–40.1%) and non-aggregated silt + clay fractions (12.2–19.0%) (Fig. 8). Moreover, the proportions of M-silt + clay, Fm-silt + clay fractions and mM-silt + clay fractions were largest in macroaggregates, free microaggregates and mM, respectively.

The distinct responses of the different fractions of the hierarchical aggregates to experimental warming were detected. Compared with CK, both WW and YW significantly decreased the proportion of Fm-POM by 18.6% and 30.5% whereas increased that of non-aggregated silt + clay fractions by 41.0% and 55.7%, respectively. Moreover, the proportion of mM-silt + clay fractions significantly increased by 31.1% in WW,

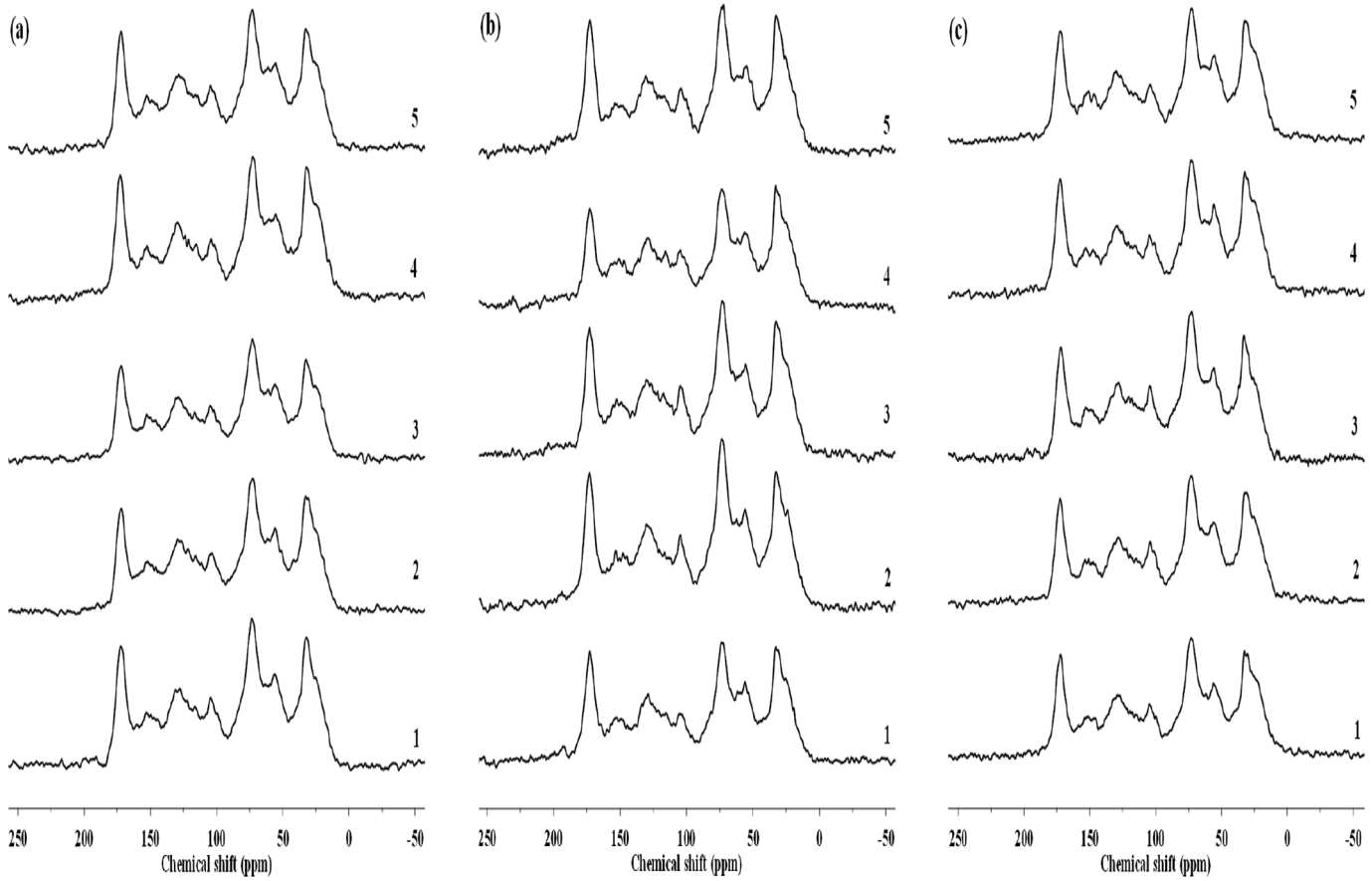

Fig. 5. Solid-state ^{13}C CPMAS NMR spectra of bulk soil samples under unwarmed control (CK) (a), winter warming (WW) (b) and year-round warming (YW) (c). The graphs listed 1 to 5 in each subplot represent the five replicates from each treatment.

whereas the proportion of M(f)POM significantly decreased by 50.0% in YW. Compared with WW, the proportion of M-silt + clay fractions was 40.5% ($p < 0.05$) higher whereas that of mM were 29.7% ($p < 0.05$) lower in YW, suggesting that mM was disrupted into M-silt + clay fractions as the intensity of warming increased.

The mean weight diameter (MWD) and geometric mean diameter (GMD) were highest in CK, followed by WW and YW (Fig. 9). The decrease in MWD and GMD implied that water erosion could be the predominant process resulting in SOC loss (Chaplot and Cooper, 2015) under warming. Significant differences were found between CK and YW for MWD and among all three treatments for GMD.

4. Discussion

Our results supported previous findings that experimental warming resulted in soil temperature increased (Li et al., 2011; Wang et al., 2014), whereas soil moisture (Li et al., 2011; Wang et al., 2014) and plant aboveground biomass (Fu et al., 2013; Zong et al., 2013) decreased in the Tibetan alpine meadows. The warming-induced soil drying was considered responsible for the reduction of aboveground biomass (Fu et al., 2013). As suggested by Peng et al. (2015), plant transpiration was the main reason for soil drying under warming in the alpine meadows of the Tibetan Plateau.

4.1. Effects of warming on SOC contents and chemical composition

The present study showed that five-year warming had no significant impacts on SOC, MBC, EOC, POC, LFOC, HFOC, HAC, FAC and HUC contents (Fig. 4). The reason could be attributed to a balance between C inputs by litter and C outputs via mineralization under warming. As has been previously reported, soil drying increased the mineralization of native (or old) soil organic matter (Enwezor, 1967; Seo et al., 2015) whereas decreased the decomposition of fresh organic matter returned into the soil (Enwezor, 1967). The destabilization of resistant phenols under warming was also been observed in the present study (Fig. 6). As a result of the counterbalance, warming caused a non-significant variation of SOC content. Our results were consistent with those of previous studies that showed that short-term (1–2 years) (Fu et al., 2012; Wang et al., 2014; Yu et al., 2014) and long-term (10 years) (Wang et al., 2014) warming did not affect SOC and MBC contents in the Tibetan alpine meadow. Moreover, by using data from a soil survey and the remote sensing vegetation index, Yang et al. (2009) indicated that SOC stocks remained relatively stable from the 1980s to 2004 in the Tibetan alpine grasslands. However, Li et al. (2011) found that short-term (2 years) warming significantly increased SOC content in the 0–5 cm layer but not in the 5–20 cm layer and that MBC at the two soil layers both increased in a Tibetan alpine meadow. Rui et al. (2011) showed that short-term (3 years) warming significantly increased MBC in the 10–20 and 20–30 cm layer but not in the 0–10 cm layer in a Tibetan alpine meadow. Therefore, warming duration may not be a crucial factor affecting SOC dynamics in the Tibetan alpine meadow. Li et al. (2011) and Rui et al. (2011) found that plant biomass increased by warming, which favored the growth of soil microbial populations and in turn the accumulation of soil microbial biomass (Zhang et al., 2015). On the other hand, the warming magnitude (Rui et al., 2011) and microbial community composition (Wang et al., 2017) were distinct among soil layers, which resulted in the different responses of SOC to warming at different soil depths.

In the present study, warming significantly increased the WSOC content of soil (Fig. 4). Thus, WSOC content may act as a sensitive

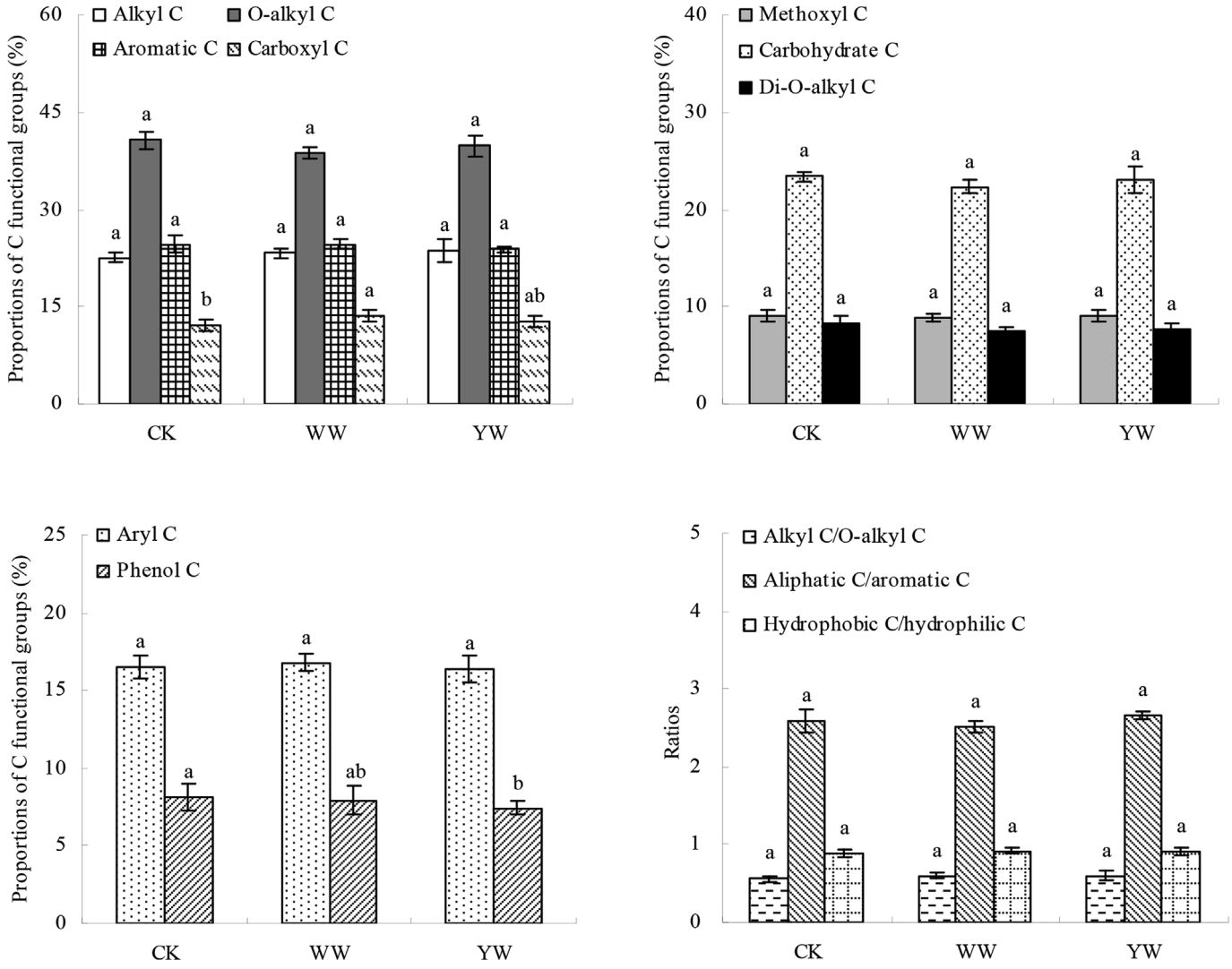

Fig. 6. Distribution of different carbon (C) functional groups in bulk soil samples obtained from solid-state ^{13}C CPMAS NMR spectra under different treatments. Vertical bars represent standard deviation of the mean (n = 5). Different letters indicate significant differences among treatments (p < 0.05). CK: unwarmed control; WW: winter warming; YW: year-round warming. Aliphatic C/aromatic C = (alkyl C + O-alkyl C)/aromatic C; Hydrophobic C/hydrophilic C = (alkyl C + aromatic C)/(O-alkyl C + carboxyl C).

indicator of future climate warming in the studied alpine meadow region. In accordance with our result, Luo et al. (2009) demonstrated that warming (2 years) significantly increased dissolved organic C (DOC) content in an alpine meadow on the Tibetan Plateau. The increase in WSOC content could be attributed to warming-induced soil drying, which could potentially accelerate the production of DOC (Tipping et al., 1999; Luo et al., 2009) and reduce their leaching (Tipping et al., 1999). Moreover, the increase of WSOC content could be due to the greater resistance to microbial degradation of WSOC produced at higher temperature (Deressa, 2015; Wang et al., 2016), which could thus decrease the loss of WSOC via biotic processes. In contrast, some studies have reported that warming (1–3 years) minimally impacted DOC content in the Tibetan alpine meadows (Rui et al., 2011; Yu et al., 2014; Zhang et al., 2015). The discrepancies could be due to numerous abiotic and biotic factors that control the responses of DOC to warming (Luo et al., 2009).

Although warming had no significant effect on SOC content, variation in the chemical composition of SOC was detected by ^{13}C CPMAS NMR spectra under climate warming (Fig. 6). In the present study, phenol C significantly decreased in soil under YW, suggesting that resistant phenol C is sensitive to climate warming. Thus, biochemical protection by the formation of resistant C compounds was not considered as a major mechanism of SOC stabilization in the alpine meadow region. Phenol C represents oxygen-substituted aromatic C from lignin units such as syringyl, guaiacyl and *p*-hydroxyphenyl (Solomon et al., 2007). A warming-induced decrease in soil moisture could create oxidative conditions (Sajedi et al., 2012). Thus, the significant decline of phenol C in YW might be attributed to the aerobic degradation of lignin by white-rot and brown-rot fungi via dehydration, demethylation, or cleavage of the β-*O*-4 bond (Derenne and Largeau, 2001), which suggested that warming stimulated microbial activity and in turn accelerated SOC decomposition in the alpine meadow. If resistant phenol C is lost over the long term, a negative effect on SOC stocks may be observed. Our result was consistent with that of Seo et al. (2015), who found that short-term (1.5 years) warming enhanced phenol oxidase activity in arctic soil. Moreover, WW significantly increased carboxyl C in soil (Fig. 6). Carboxyl C is mainly derived from carboxyl acid, amide and ester (Mathers and Xu, 2003). The significant increase in carboxyl C under WW might be related to the oxidation of lignin side-chains, aromatic rings and polysaccharides, and the accumulation of fatty acids during SOM degradation (Zech et al., 1997). Using a 180-day incubation experiment, Wang et al. (2016) found that carboxyl C of HA and HU tended to increase with increasing temperature, which was similar to the result of the present study. It has been previously reported that substrate quality was a major determinant of soil organic matter quality (Zech et al., 1997). The quality of soil

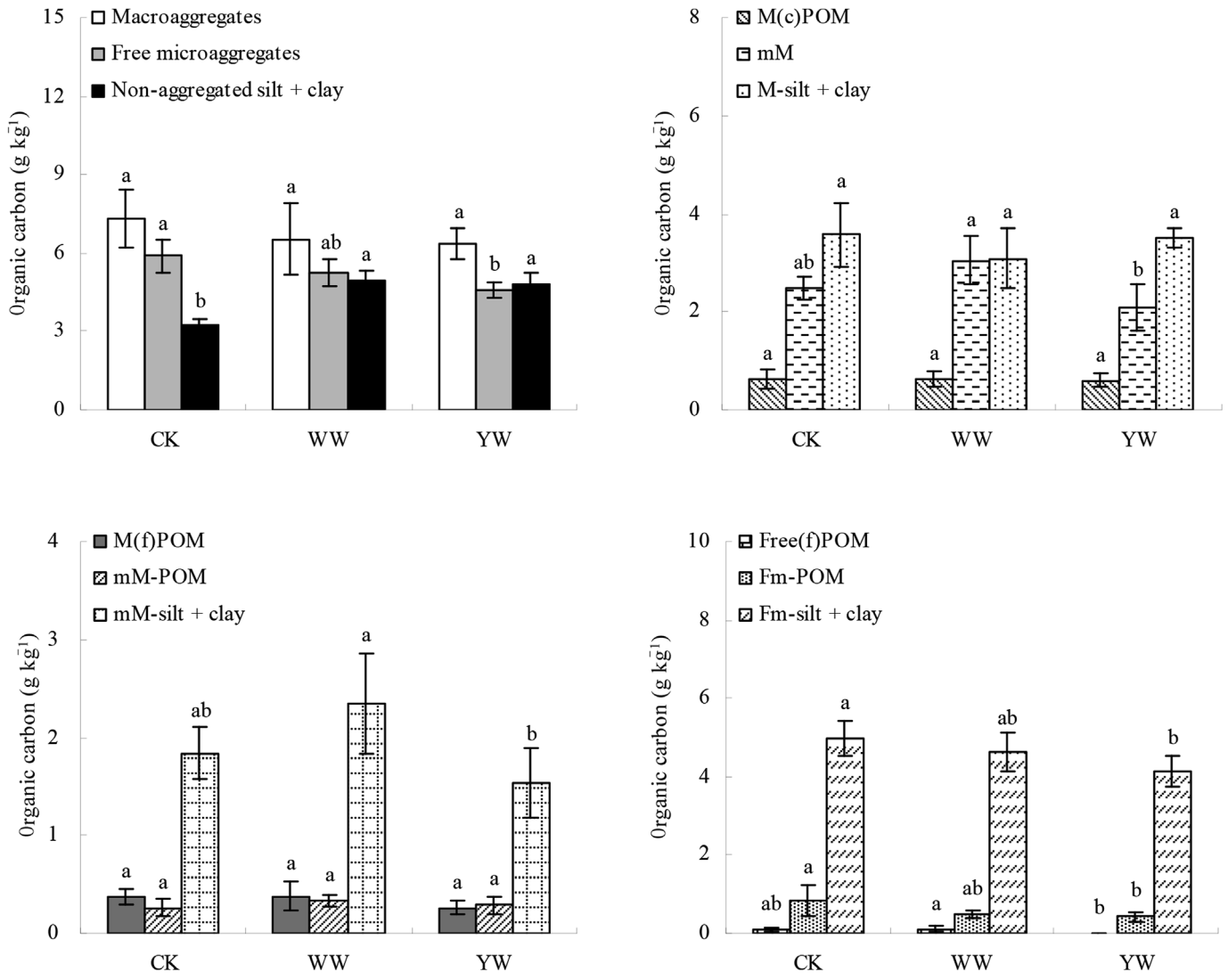

Fig. 7. Organic carbon contents of hierarchical aggregates in soil under different treatments. Vertical bars represent standard deviation of the mean (n = 5). Different letters indicate significant differences among treatments (p < 0.05). CK: unwarmed control; WW: winter warming; YW: year-round warming. For other abbreviations, see Fig. 2.

organic matter could be related to the physiological adaptation of plants to environmental conditions, which could be preserved as an imprint in soil organic matter (Campo and Merino, 2016). Therefore, further studies are necessary to examine the response of substrate quality to climate warming, which might clarify the mechanisms of variation in SOC chemical composition.

4.2. Effects of warming on C associated with hierarchically organized soil aggregates and aggregate stability

The result of the present study indicated that the experimental soil contained a high proportion of macroaggregates (Fig. 8) and thus exhibited a good soil structure. The distribution of different size fractions of aggregates in soil suggested that warming led to larger aggregates broken down into non-aggregated silt + clay fractions, partly because of warming-induced soil drying that increased soil erosion and runoff and prevented soil aggregation and structural development (Bronick and Lal, 2005). Fm-POM and M-silt + clay in WW and Fm-POM and M (f)POM in YW contributed more to the disruption of larger aggregates. A previous study showed that Fm-POM could act as a cementing agent for the formation of stable microaggregates (Watteau et al., 2012). In contrast, WW promoted the formation of mM-silt + clay fractions, which could act as binding agents for macroaggregates (Yin et al., 2015) and in turn could suppress the disruption of macroaggregates. However, Cheng et al. (2011) found that warming (9 years) did not significantly affect the size distribution of soil aggregates in a North America tallgrass prairie.

As proposed by previous studies, organic C stored in different size fractions was primarily controlled by the amount of each size class of aggregate (Cheng et al., 2011). In the present study, the decrease in organic C content with decreasing aggregate size (Fig. 7) supported the aggregate hierarchy model (Six et al., 2004; Kong et al., 2005). According to this model, microaggregates are bound together into macroaggregates by organic binding agents (Tisdall and Oades, 1982; Six et al., 2004). Thus, the aggregate hierarchy is essentially an increase of organic matter content with increasing size class (Kong et al., 2005; John et al., 2005). In addition, the present study showed that silt + clay fractions contained more organic C than POM inside both macro- and micro-aggregates (Fig. 7), suggesting that organic C in macro- and micro-aggregates was mainly preserved by the inorganic binding of silt + clay fractions. Similarly, Smith et al. (2014) found that minerals rather than POM played a key role in the formation and stability of macroaggregates in highly weathered tropical soils.

SOC stabilization depended, in part, on its distribution in aggregates (Nie et al., 2014). According to the hierarchical theory of aggregate formation, macroaggregates could be formed by microaggregates

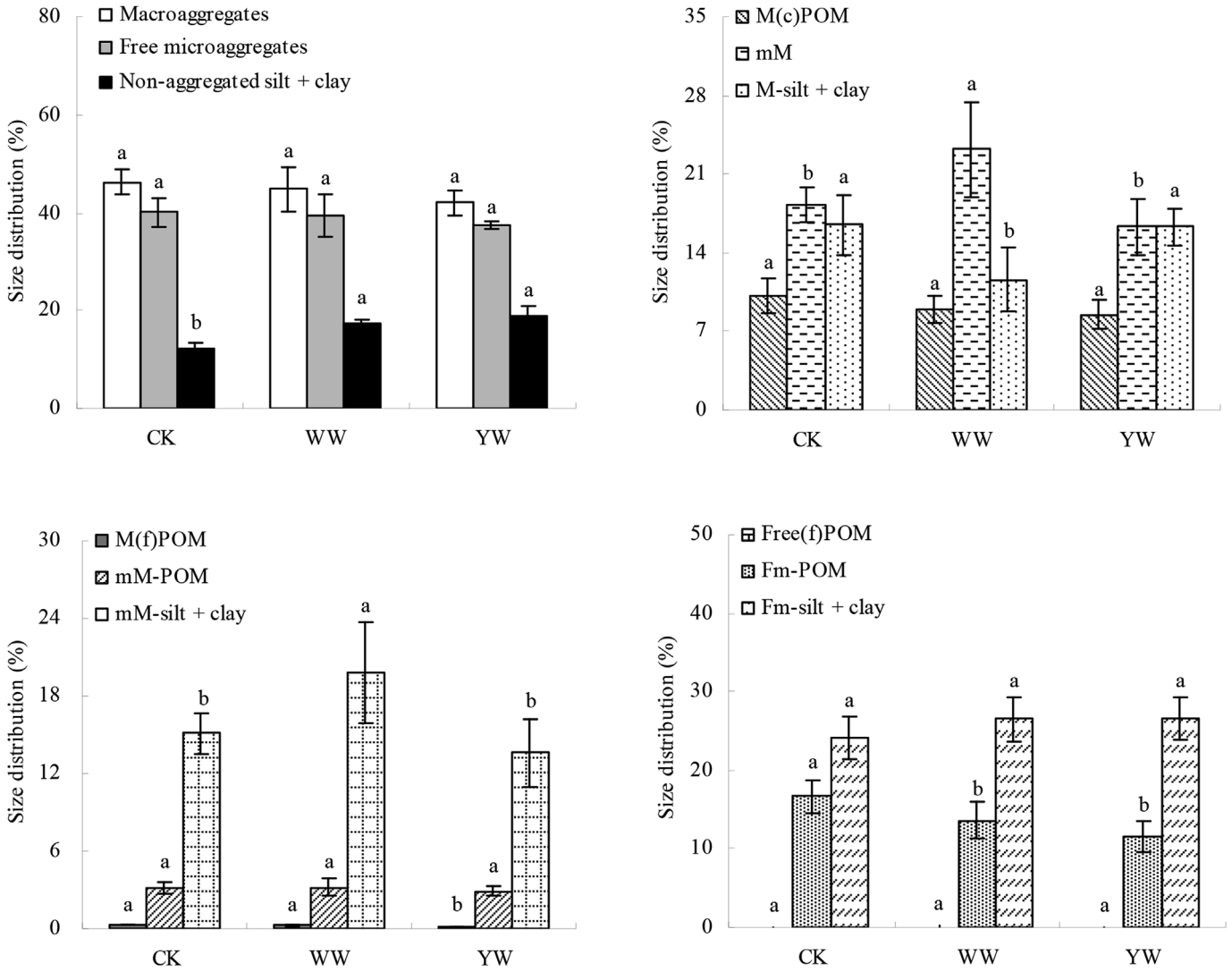

Fig. 8. Size distribution of hierarchical aggregates in soil under different treatments. Vertical bars represent standard deviation of the mean (n = 5). Different letters indicate significant differences among treatments (p < 0.05). CK: unwarmed control; WW: winter warming; YW: year-round warming. For other abbreviations, see Fig. 2.

combined with plant-derived (i.e., new) organic matter (Six et al., 2004). Consequently, macroaggregates contained more plant-derived C than other size classes of soil aggregates and were, in turn, less stable (Zhong et al., 2017). However, the present study showed that experimental warming had no significant effect on macroaggregate-associated organic C (Fig. 7). The lack of change in macroaggregate-associated C could be due to a balance between the inputs and outputs of C in which organic C loss was offset by litter C inputs under short-term warming, which suggested that warming prevented SOC accumulation in the alpine meadow.

Although the proportion of free microaggregates was not significantly affected by experimental warming, organic C associated with free microaggregates significantly decreased under YW (Fig. 7). Thus, physical protection within stable aggregates was not a major mechanism of SOC stabilization in the alpine meadow. The free microaggregate-associated C may be a sensitive indicator of future climate warming in this region. One probable reason was that free microaggregates contained less plant-derived organic matter (Cheng et al.,

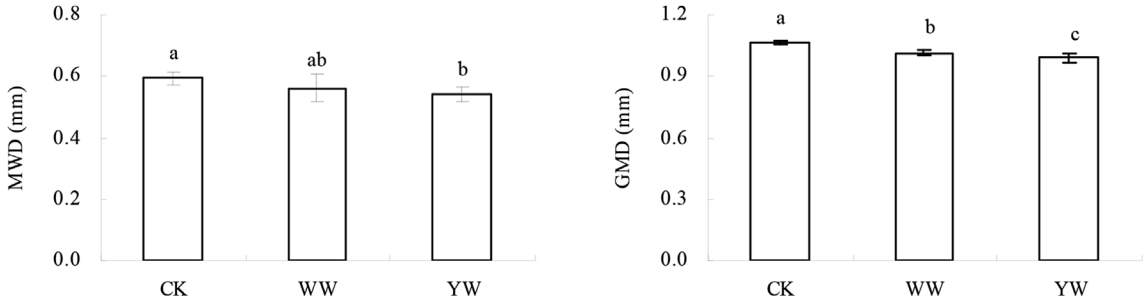

Fig. 9. Mean weight diameter (MWD) and geometric mean diameter (GMD) of water-stable aggregates in soil under different treatments. Vertical bars represent standard deviation of the mean (n = 5). Different letters indicate significant differences among treatments (p < 0.05). CK: unwarmed control; WW: winter warming; YW: year-round warming.

2011) and free microaggregate-associated C was more temperature sensitive (Tao and Song, 2013; Sandeep and Manjaiah, 2014; Ghosh et al., 2016) than that of macroaggregates, resulting in more C loss than offset by litter C inputs for free microaggregates under warming. Furthermore, we found that Fm-POM and Fm-silt + clay fractions were mainly responsible for the decrease of free microaggregate-associated C under YW (Fig. 7). Previous studies have demonstrated that physical occlusion of SOC within microaggregates enhanced their protection from microbial breakdown and ultimately facilitated long-term C sequestration (Tisdall and Oades, 1982; Verchot et al., 2011). Therefore, the decrease in free microaggregate-associated C implied that climate warming might influence long-term SOC storage in the alpine meadows of the Tibetan Plateau.

In this study, we found that warming significantly increased C associated with non-aggregated silt + clay fractions (Fig. 7), suggesting a preservation of C in non-aggregated organic-mineral fractions. It has been reported that C associated with non-aggregated silt + clay fractions was more stable than C associated with macro- and micro-aggregates (Six et al., 2004; Saha et al., 2010). Thus, our results suggested that non-aggregated silt + clay fractions are important for the stabilization of SOC under climate warming via chemical protection by mineral association. Superficially, the increase in mineral-associated C appeared to maintain SOC under warming scenarios. However, as shown in Fig. 7, non-aggregated silt + clay fractions contained less C than macro- and micro-aggregates. The increase of C-depleted non-aggregated silt + clay fractions might may indicate the potential damage of C-rich macro- and micro-aggregates. In fact, a decrease of C associated with macro- and micro-aggregates was observed in the present study (Fig. 7). Therefoer, the increase of C-depleted non-aggregated silt + clay fractions following the decrease of C-rich macro- and micro-aggregates caused by warming might not be beneficial for the long-term accumulation of SOC in the alpine meadow of Tibet.

Our results suggested that climate warming negatively impacted soil aggregate stability based on a significant decrease in MWD and GMD (Fig. 9). Thus, MWD and GMD could also act as sensitive indicators of future climate warming in the Tibetan alpine meadow. Decreasing aggregate stability led to a significant increase in the proportion of non-aggregated silt + clay fractions. Additionally, the decline of aggregate stability made organic matter physically accessible to soil microbes (Conant et al., 2011), which caused macro- and free microaggregate-associated C to be reduced. The decrease in aggregate stability could be attributed to variations in the amount and quality of litter returned to the soil under warming. On the one hand, the low input of aboveground litter resulted from warming-induced soil drying prevented soil aggregation and increased soil erosion (Bronick and Lal, 2005), which in turn caused the aggregate stability to decline. On the other hand, hydrophobic organic matter components have been reported to enhance the water stability of soil aggregates (Piccolo and Mbagwu, 1999). The decline in aggregate stability under warming might thus be partly explained by a significant decrease in hydrophobic phenolic functional groups and a significant increase in hydrophilic carboxylic functional groups (Fig. 6). Previous studies have also found that phenols could act as binding agent to increase soil aggregation (Martens, 2000; Bronick and Lal, 2005). As indicated above, the quality of soil organic matter was closely related to the biochemical composition of plant residues returned to the soil. Therefore, further studies are needed to examine the relationship between soil aggregate stability and substrate quality under climate warming. In accordance with our present result, Rillig et al. (2002) showed that experimental warming significantly decreased soil aggregate water stability in an annual grassland of California because of a reduction in immunoreactive glomalin. Moreover, in laboratory incubation experiments, some researchers also found that the water stability of aggregates in soil declined with increasing temperature (Annabi et al., 2004; Wang et al., 2016), which were also similar to our present result. However, in contrast to our finding, Cheng et al. (2011) found that warming (9 years) did not significantly affect soil aggregate stability in a North America tallgrass prairie. This discrepancy might occur because soil aggregate stability depends on many factors such as temperature, moisture, soil properties, and plant species (Bronick and Lal, 2005).

4.3. Possible effects of long-term warming on SOC stocks in the Tibetan alpine meadow

Because of high background soil C levels, it is difficult to detect changes in SOC stocks in response to climate change in the short term (Kirschbaum, 2006; Song et al., 2012). Therefore, it was not surprising that total SOC contents appeared constant even after five years of experimental warming in the present study. However, we observed some significant changes in plant biomass, SOC fractions contents, SOC chemical compositions, and soil structural stability over YW, and with prolonged time, these might resulted in a change in SOC stocks. In this study, plant aboveground biomass significantly decreased under YW, meaning that the C inputs to the system were reduced. At the same time, WSOC content significantly increased under YW, which we would expected to stimulate soil microbial activity and in turn increase organic matter decomposition over the long term. In fact, some resistant C compounds (phenol C), stable C fractions (free microaggregate-associated C) and aggregate stability in soil have been observed to be significantly reduced under YW. As discussed above, these reductions would not be beneficial for the long-term accumulation of SOC. Therefore, given time, YW would most likely negatively affect SOC stocks in the Tibet alpine meadow.

5. Conclusions

This study showed that short-term warming reduced plant aboveground biomass but had no significant influence on SOC, MBC, EOC, POC, LFOC, HFOC, HAC, FAC and HUC contents. In contrast, warming significantly increased WSOC content and affected the chemical composition of SOC with decreasing phenol C and increasing carboxyl C. Moreover, organic C associated with free microaggregates decreased whereas organic C associated with non-aggregated silt + clay fractions significantly increased. For hierarchically organized soil aggregates, non-aggregated silt- and clay-sized fractions noticeably increased, which resulted in MWD and GMD of water-stable aggregates declined under warming. The results suggested that climate warming did not affect total soil C stocks but positively impacted WSOC, carboxyl C and non-aggregated silt- and clay-associated C and negatively impacted phenol C, free microaggregates-associated C and aggregate stability. These variables might act as sensitive indicators of climate warming in the alpine meadow of Tibet, which in turn affect grassland ecosystem C fluxes in response and feedback to further climate change. The chemical protection by mineral association, rather than physical and biochemical protections, was the primary mechanism for the stabilization of SOC under climate warming in the alpine meadow.

Given that the negative effect caused by C outputs under warming could be partly offset by plant litter C input, the alpine meadow in this region should be specifically protected from anthropogenic disturbance (such as overgrazing) and sustainable management practices (such as rational fertilization) should be developed to maintain high grassland productivity for addressing future climate warming. Further study is required to determine the long-term fates of SOC stocks under warming and develop models that consider SOC fractions to accurately forecast the responses of terrestrial ecosystems to global climate change.

Acknowledgements

This work was supported jointly by the National Natural Science Foundation of China (Nos. 31470506 and 41471196), the National Key Research Program of China (No. 2016YFC0500202), and the Program for Kezhen Distinguished Talents in Institute of Geographic Sciences

and Natural Resources Research (No. 2013RC102). We would like to thank the editors and anonymous reviewers for their valuable comments and suggestions.

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